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ORIGINAL ARTICLE

Usefulness of therapeutic drug monitoring of rilpivirine and its relationship with virologic response and resistance in a cohort of naive and pretreated HIV-infected patients

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Aims: The purpose of this study was to assess the antiviral activity of the rilpivirine/emtricitabine/tenofovir disoproxil fumarate combination and to describe the pharmacokinetics of rilpivirine and its association with resistance in clinical routine.

Methods: A retrospective multicentre cohort study was performed in both naive and pretreated HIV patients receiving the once-daily rilpivirine/emtricitabine/tenofovir disoproxil fumarate regimen. Immuno-virologic and resistance data, and rilpivirine plasma trough concentrations were collected over the follow-up. Statistical analyses were performed to evaluate the relationship between rilpivirine pharmacokinetics and virological response. Receiver operating characteristic (ROC) curve analysis was performed to determine the best target rilpivirine trough concentration.

Results: Overall, 379 patients were included. After a median follow-up of 28 months, 26% of patients discontinued mainly due to toxicity and the virological success rate was 65.7%. Virological failure occurred in 5% of patients. A significant proportion of patients with HIV-RNA > 40 copies/mL displayed rilpivirine plasma trough concentrations below the currently used 50 ng/mL efficacy threshold at both M6 (28%) and M12 (31%), in agreement with a significant lower median rilpivirine plasma trough concentration compared with patients virologically suppressed. Half of the patients with virologic failure who acquired rilpivirine resistance mutations had at least one suboptimal rilpivirine trough concentration. The optimal target for rilpivirine trough concentration was 70 ng/mL (sensitivity 75.4%; specificity 61.5%).

Conclusions: This study shows the impact of rilpivirine plasma trough concentration on both virological response and the emergence of rilpivirine mutations. Moreover, our results suggest that a higher target of rilpivirine trough concentration could be proposed in clinical practice.

KEYWORDS

antiretroviral therapy, HIV, pharmacodynamics, pharmacokinetics, rilpivirine

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1 | INTRODUCTION

The combination of rilpivirine (RPV), a second-generation non-nucleoside reverse transcriptase inhibitor (NNRTI) and two nucleos(t)ide reverse transcriptase inhibitors (NRTI), emtricitabine (FTC) and tenofovir disoproxil fumarate (TDF) is commonly used for the treatment of HIV-1 infection both in naive and treatment-experienced patients. This combination is available in a co-formulated single tablet regimen (STR) (RPV/FTC/TDF 25/200/245 mg) taken once daily (QD), which improves treatment adherence and maximizes the success of antiretroviral (ARV) treatment.[1,2] The phase III studies proved that RPV/FTC/TDF had an antiviral efficacy similar to efavirenz (EFV)-based regimens in naive patients (NP) with a plasma HIV-1 viral load (VL) at baseline ≤100,000 copies/mL.[3–5] The efficacy was also demonstrated in patients virologically suppressed switching from either EFV/FTC/TDF or a protease inhibitor-based regimen to RPV/FTC/TDF.[6,7] Moreover, RPV/FTC/TDF provides major advantages in terms of tolerance compared to other STRs. Indeed, data from phase III studies showed that side effects, such as neurological symptoms with a grade ≥ 2, were significantly less frequent with RPV-based regimens than with EFV (16% *vs* 31%). Moreover, discontinuations due to adverse effects occurred at a lower rate with RPV.[3,4] RPV displays a different pattern of resistance mutations than first-generation NNRTIs due to a greater flexibility and a specific interaction with the reverse transcriptase binding site.[8] It is making RPV an appropriate alternative treatment for patients failing first NNRTI regimens. Nonetheless, RPV is not recommended for patients harbouring a baseline VL > 100,000 copies/mL, related to higher rates of virological failure (VF) and resistance selection.[4,9] Overall, the most frequently selected mutations impacting NNRTI and NRTI were E138K (72%) and M184I (69%), respectively.[10]

The pharmacokinetics (PK) properties of RPV, such as both the food- and pH-dependent intestinal absorption and the intense hepatic metabolism through the cytochrome P-450 3A4 (CYP3A4), enhance the variability of RPV exposure, and might compromise the antiviral efficacy. Population pharmacokinetic (Pop-PK) models developed in cohorts of unselected HIV-1-infected adult patients showed a moderate interpatient variability of RPV PK parameters but a lower apparent volume of distribution associated with a shorter terminal elimination half-life ($t_{1/2}$) than reported in the Summary of Product Characteristics.[11,12] This suggests that, in routine clinical practice, patients may be at greater risk of suboptimal exposure than expected, which may increase the risk of developing HIV-resistant variants as demonstrated in vitro and in vivo with other ARV treatments.[13,14] In phase III studies, a strong relationship between RPV trough plasma concentration (C_{trough}) and efficacy was established whatever the level of baseline VL (≤100,000 copies/mL or >100,000 copies/mL). Patients with an RPV C_{trough} > 50 ng/mL have an 80% probability of achieving virological success.[15] Therefore, an efficacy threshold of 50 ng/mL is currently recommended in clinical practice as a minimal target RPV C_{trough} to increase the probability of virological response in clinical practice.[16]

However, studies combining PK and efficacy of RPV are lacking in real-life contexts.[17–22] Hence, the present study assessed the antiviral activity of the RPV/FTC/TDF regimen and described the association of RPV exposure with virologic response and resistance in a cohort of NP and treatment-experienced patients (TEP) over 3 years of follow-up.

What is known about this subject

- Rilpivirine is widely prescribed for the treatment of HIV-1 infection both in induction or maintenance strategies.
- The trough plasma concentration of rilpivirine is highly correlated with virological response.
- An important inter-individual rilpivirine PK variability is described in clinical trials as well as in clinical routine that could affect both efficacy and safety of the treatment.

What this study adds

- A significant proportion of patients with HIV-RNA > 40 copies/mL displayed rilpivirine plasma C_{trough} below the currently used 50 ng/mL efficacy threshold.
- A significant impact of rilpivirine plasma C_{trough} on virological response and a relationship with the emergence of rilpivirine-associated mutations.
- A higher efficacy target for rilpivirine plasma C_{trough} should be considered to obtain virological response.

2 | PATIENTS AND METHODS

2.1 | Patients

Ambulatory patients who had initiated RPV/FTC/TDF regimen between November 2012 and November 2015 in the University hospital of Marseille and Bichat-Claude Bernard (Paris) were included retrospectively in this observational study. The date when

RPV/FTC/TDF therapy was started was the baseline for each patient. Patients were followed up for a period of 3 years after RPV/FTC/TDF initiation. This study was carried out in compliance with the International guidelines for human research protection (Declaration of Helsinki and ICH-GCP).[23,24] Patients provided written informed consent for the use of their medical records on Nadis® (Fedialis, Marly-Le-Roi, France; electronic medical record for patients infected by HIV, HBV and HCV, approved by the French *Commission Nationale Informatique et Liberté*; registration number: 2001/762876/nadiscnil.doc).

2.2 | Clinical and biological assessments

Baseline data of the patients, including demographic characteristics, therapeutic status, prior ARV therapy, reasons for switch, CD4 and VL, were collected from Nadis®. Data were described by median and range or frequency (%). Clinical follow-up data, including VL and CD4, were collected at routine follow-up visits at 6 months (M6), M12, M24 and M36 ± 2 months and were reported as median and interquartile range (IQR). The proportion of treatment interruptions and their reasons were investigated. Virological response was defined as the proportion of individuals who achieved viral suppression (plasma VL ≤ 40 copies/mL). The response rates were calculated using the intention-to-treat principle, whereby all missing data were treated as failures. The proportion of virological failures (VF), defined as a confirmed plasma VL > 40 copies/mL, the resistance profile associated to VF, the number and amplitude of viral blips (transient VL > 40 copies/mL preceded and followed by a VL ≤ 40 copies/mL) in patients virologically suppressed, and the CD4 counts were also collected. NNRTI and NRTI-resistance mutations were obtained from the last genotype available prior to starting RPV/FTC/TDF treatment and from the cumulative genotype. In the case of VF, NNRTI and NRTI-resistance mutations were also collected if genotype was available. Resistance mutations were defined and interpreted according to the ANRS (Agence Nationale de Recherche sur le Sida et les hépatites virales) drug resistance algorithm (http://hivfrenchresistance.org, version 27).

2.3 | RPV pharmacokinetics

Blood samples were collected at steady state, at various registered post-dose times ranging from 1 to 31 hours and over the course of the RPV/FTC/TDF treatment. C_{trough} was defined as the concentration at 24 hours ± 6 hours after the last drug intake. RPV plasma concentrations were determined by a sensitive and validated reverse-phase ultra-performance liquid chromatography coupled to a tandem mass spectrometry method, as previously described.[25] The assay was validated over a calibration range of 10 to 5,000 ng/mL with a lower limit of quantification of 10 ng/mL. Both accuracy and precision were less than 15%.

2.4 | Statistical analysis

Intra-individual variability was evaluated by averaging the coefficients of variation (CV) (defined as the ratio of the standard deviation to the mean) of all the available RPV C_{trough} from each individual patient throughout the follow-up period. Inter-individual variability was calculated using the CV for the mean of available RPV C_{trough} from each subject.

Comparison of RPV C_{trough} between patients with VL ≤ 40 and > 40 copies/mL was analysed by the non-parametric Mann–Whitney *U*-test. The percentage of patients with RPV C_{trough} below the threshold of 50 ng/mL was compared between both populations by Fisher exact test. Analyses were performed at M6, M12, M24 and M36.

Optimal target for the RPV C_{trough} was determined using receiver operating characteristic (ROC) curve analysis. Area under the curve (AUC), sensitivity and specificity values were used for the evaluation of the optimal cut-off.

Statistical analyses were performed using R software v 3.5.3 (https://cran.r-project.org/). A *P*-value of <0.05 was considered statistically significant.

3 | RESULTS

3.1 | Demographic and pharmacodynamic data at baseline

A total of 65 NP and 314 TEP were included in the study of which 270 TEP (86%) had an undetectable baseline VL. The population consisted of 71% male patients. The median age at inclusion was 44 years (range: 19–82 years). Baseline characteristics of NP and TEP are shown in Table 1. Sex ratio and median age were similar between NP and TEP. The median number of ARV regimens received by TEP before RPV/FTC/TDF therapy was four and boosted PI was predominant in the previous regimens. Simplification was the main reason for switching, followed by adverse effects.

Among the 283 patients (74.7%) with an available genotype, 93 (32.9%) presented resistance mutations at baseline (Table 1). Among them, a total of 64 patients (68.8%), 9 NP and 55 TEP, harboured NNRTI resistance mutations: 45 patients with *n* = 1 mutation, 13 (*n* = 2), 5 (*n* = 3) and 1 (*n* = 4). Among them, 16 patients (14 TEP and 2 NP) presented specific mutations conferring resistance to RPV. Moreover, based on the cumulative genotype, five more TEP showed mutations conferring resistance to RPV. The NNRTI mutations were: A98S (*n* = 12), K103N (*n* = 9), V179I (*n* = 9), V90I (*n* = 6), V179VI (*n* = 5), A98G (*n* = 3), K101R (*n* = 3), G190A (*n* = 3), K103H/N/S/T (*n* = 2), V106I (*n* = 2), H221H (*n* = 2), P236M (*n* = 2), A98AE (*n* = 1), A98AG (*n* = 1), K101Q (*n* = 1), K101KR (*n* = 1), K101KC (*n* = 1), K103R (*n* = 1), K103KN (*n* = 1), V106VI (*n* = 1), V108I (*n* = 1), V108VI (*n* = 1), V179I/L/M/T (*n* = 1) and M230P (*n* = 1). The mutations conferring resistance to RPV were: E138A (*n* = 6), V179L (*n* = 5), M230MI (*n* = 3), E138K (*n* = 2), K101E (*n* = 1), V179D (*n* = 1),

TABLE 1 Patients characteristics at baseline

Parameters median (range) or *n* (%)	Naive population (NP) (*n* = 65)	Treatment-experienced population (TEP) (*n* = 314)
Male gender	53 (81.5%)	215 (68.5%)
Age, years	37 (19–70)	44 (19–82)
CD4, cells/mm^3	480 (24–1609)	602 (20–2351)
Nadir CD4, cells/mm^3	373 (24–1609)	247 (1–1275)
Plasma HIV RNA, copies/mL	15,318 (40–3141 000)	40 (40–285 022)
% ≤ 40 copies/mL	3 (4.6%)	270 (85.9%)
Co-infection		
HBV	3 (4.6%)	31 (9.9%)
HCV	3 (4.6%)	32 (10.2%)
HBV and HCV	–	4 (1.3%)
Number of prior ARV regimens	–	4 (2–26)
Time since first antiretroviral medication, years	–	6 (0.08–25.6)
Last ART treatment		
2 NRTIs + 1 PI/r	–	176 (56.1%)
2 NRTIs + 1 NNRTI	–	103 (32.8%)
2 NRTIs + II	–	22 (7.0%)
Other	–	13 (4.1%)
Reasons for switching		
Simplification	–	166 (52.8%)
Adverse effects	–	106 (33.8%)
Virologic failure	–	10 (3.2%)
Other	–	25 (8.0%)
Not available	–	7 (2.2%)
Available genotype	54 (83.1%)	229 (72.9%)
HIV-RNA not amplifiable	7 (10.8%)	20 (6.4%)
Not available	4 (6.2%)	65 (20.7%)
WT genotype	43 (79.6%)	147 (64.2%)
Presence of resistance mutations	11 (20.4%)	82 (35.8%)
NNRTI mutations only	7 (13%)	35 (15.3%)
NRTI mutations only	2 (3.7%)	27 (11.8%)
NNRTI + NRTI mutations	2 (3.7%)	20 (8.7%)

PI/r: boosted protease inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; NRTI: nucleoside/nucleotide reverse transcriptase inhibitor; II: integrase inhibitor; HBV: Hepatitis B virus; HCV: Hepatitis C virus; WT: wild type.

Y181YC (*n* = 1), Y188L (*n* = 1), Y181C (*n* = 1) and K103N+ L00I (*n* = 1). The K103N mutation was present in 13 patients, always in association with other mutations except in one patient.

Among the patients with resistance mutations at baseline, 51 patients (54.8%) harboured NRTI resistance mutations. Most of them (*n* = 27) harboured only one NRTI mutation and other patients had between two and seven NRTI mutations: 2 (*n* = 8), 3 (*n* = 3), 4 (*n* = 8), 5 (*n* = 2), 6 (*n* = 1) and 7 (*n* = 2). The NRTI mutations identified were: M184V (*n* = 15), M41L (*n* = 13), D67N (*n* = 13), V118I (*n* = 8), T215Y (*n* = 8), K70R (*n* = 6), K219Q (*n* = 5), T69N (*n* = 4), M184V/I (*n* = 4), L210W (*n* = 4), T215F (*n* = 3), K70KR (*n* = 2), K70KT (*n* = 2), L74I (*n* = 2), T215N (*n* = 2), T215S (*n* = 2), K19E (*n* = 2), E44ED (*n* = 1), T69N/S (*n* = 1), T69S (*n* = 1), M41ML (*n* = 1), M184MV (*n* = 1), M184MIV (*n* = 1), L210G (*n* = 1), T215C (*n* = 1), T215L (*n* = 1), T215TS (*n* = 1), T215CDG (*n* = 1), T215TFIS (*n* = 1) and T215K (*n* = 1).

3.2 | Clinical follow-up

The median follow-up duration was 28 months. Thirteen patients were lost to follow-up at M6 (*n* = 4), M12 (*n* = 2), M24 (*n* = 6), M36 (*n* = 1), and 15 patients were transferred to another facility (4 at M6, 3 at M12, 3 at M24 and 5 at M36). Of the 351 patients remaining (55 NP and 296 TEP), 91 (26%) including 20 NP (36.4%) and 71 TEP

(23.9%), discontinued the treatment at M6 (*n* = 14), M12 (*n* = 16), M24 (*n* = 37) and M36 (*n* = 24). The most frequent cause of discontinuation in both populations was toxicity (*n* = 43, 47%). Among adverse event (AE)-related interruptions, neuropsychiatric and renal side effects occurred in 32.6% (*n* = 14) and 20.9% (*n* = 9) of patients, respectively. The other AE-related interruptions were gastro-intestinal (*n* = 2), osteoarticular (*n* = 3), dyslipidaemia (*n* = 3), hepatic (*n* = 2), cutaneous (*n* = 1) and unknown (*n* = 9). The other reasons leading to treatment interruption were VF (20.9%), simplification (13.2%), pregnancy (10.9%), patient decision (2.2%), unknown (4.4%) and one TEP died from bronchial cancer (not related to the treatment).

3.3 | Virological outcomes

Virologic success was achieved in 88.6%, 84.2%, 75.2% and 65.7% of patients at M6, M12, M24 and M36, respectively (Figure 1). Among TEP with plasma VL detectable at baseline (*n* = 44), the proportion of patients in virologic success was 84.1%, 61.4%, 52.3% and 38.6% at M6, M12, M24 and M36, respectively. Regarding initially virologically-suppressed TEP patients (*n* = 270), the proportions were 92.6%, 88.9%, 81.1%, 74.8%, respectively.

Seven TEP (2.2%) and four NP (6.2%) had isolated viral blip during the treatment. One TEP presented another viral blip 6 months after the first blip episode. The median viral blip amplitude was 222 copies/mL (range 57–1,087 copies/mL). All these patients maintained virologic suppression until the end of the follow-up period. The CD4 count increased from a median of 577 cells/mm^3 (IQR: 411–770) at baseline to 591 cells/mm^3 (IQR: 450–800), 610 cells/mm^3 (IQR: 479–840), 651 cells/mm^3 (IQR: 492–826), 681 cells/mm^3 (IQR: 508–840) at M6, M12, M24 and M36, respectively.

Nineteen patients (5%) discontinued the regimen because of VF, in similar proportions between NP (6.1%) and TEP (4.8%), respectively at M6 (4 TEP), M12 (2 NP; 5 TEP), M24 (1 NP; 6 TEP) and M36 (1 NP). Among the 15 TEP who failed the regimen, 6 (40%) were virologically suppressed at baseline.

3.4 | Resistance analysis

Among the 19 patients with VF, HIV genotyping was performed and available for 17 patients. The details of the resistance profile and pharmacological data of these patients at baseline and at the time of failure are summarized in Table 2. Eight (47%) patients had acquired RPV mutations at failure, namely E138K (*n* = 3), M230L (*n* = 2), E138Q (*n* = 1), E138KQ (*n* = 1), Y181C (*n* = 1), Y181I (*n* = 1), Y188YFL (*n* = 1) and M230I (*n* = 1). Among these eight patients, a suboptimal RPV plasma C_{trough} was observed once for one patient and twice for three patients during the follow-up. Among the 21 patients with pre-existing RPV-associated resistance mutations, 12 were virologically suppressed at M36, one was lost to follow-up, seven discontinued the treatment and only one experienced a virologic failure at M5, while displaying RPV C_{trough} above 50 ng/mL. However, this patient had the combination K103N + L100I at baseline, which conferred a complete resistance to RPV. Only three out of the 13 patients harbouring the K103N mutation at baseline failed the regimen, including the patient with the K103N + L100I mutations.

3.5 | RPV pharmacokinetics

A total of 779 RPV plasma concentrations were measured in the population and on average two samples per patient were available. The median values of RPV concentrations were 99 ng/mL (IQR: 68–143 ng/mL) and 101 (IQR: 68–155 ng/mL) in NP and TEP, respectively. Among these samples, 662 RPV C_{trough} were available in 343 patients, respectively in 55 NP and 288 TEP. The analysis of RPV C_{trough} values is presented in Table 3. Over the follow-up period, suboptimal RPV C_{trough} were observed for 64 patients (18.7%), which was similar in NP (18.2%) and TEP (18.8%). The intra- and inter-subject variabilities were 27% and 54%, respectively and similar between NP and TEP.

At M6, the median (IQR; *n*) RPV C_{trough} was significantly lower in patients with VL > 40 copies/mL compared with patients virologically

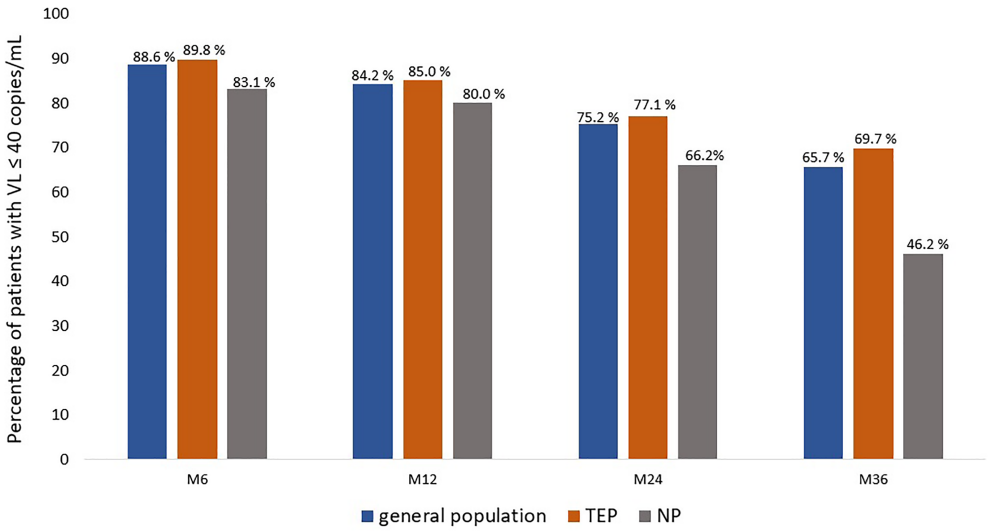

FIGURE 1 Virologic suppression at 6 months (M6), 12 months (M12), 24 months (M24) and 36 months (M36) for pretreated patients (TEP) and naive patients (NP)
Percentage of patients with viral load (VL) ≤ 40 copies/mL at M6, M12, M24 and M36 for general population (blue), TEP (orange) and NP (grey). N represent the number of patients reaching VL ≤ 40 copies/mL

TABLE 2 Resistance profile and pharmacological data of the 19 patients with virologic failure

		Previous cART before the switch	VL at baseline copies/mL	VL at failure copies/mL	Time of VF month	RT mutations at baseline	RT mutations at failure	RPV C_{trough} ng/mL
1	NP	–	6971	169 800	10	None	K103KN, **E138KQ, Y188YFL**	**23** (M3); **23** (M10)
2	NP	–	15 804	40	10	A98S	A98S	97 (M2); 69 (M3); 106 (M8)
3	NP	–	286 890	212	19	K103R, V179I	K65T, K103R, V179I, **Y181C, M230L**	260 (M14)
4	NP	–	NA	498	27	None	None	84 (M7)
5	TEP	Atazanavir/r TDF/FTC	285 022	236	3	None	None	198 (M1); 68 (M3)
6	TEP	Lopinavir/r Lamivudine/abacavir	153 340	145 600	9	D67N, T69DN, K70R, V118I, M184V, T215F, K219Q	D67N, T69D, K70R, V118I, V179I, **Y181I** M184V, T215F, K219Q	62 (M1); 82 (M5); 56 (M9)
7	TEP	Darunavir/r TDF/FTC	40	521	3	None	None	155 (M1)
8	TEP	Darunavir/r TDF/FTC	81	66	5	NA	T69DN	104 (M5)
9	TEP	Raltegravir TDF/FTC	40	49	17	None	None	52[a] (M6)
10	TEP	Darunavir/r TDF/FTC	58	94	16	None	None	70 (M9)
11	TEP	Fosamprenavir/r TDF/FTC	8465	803	12	None	V106A, **E138K,** M184V, L210W, T215D	**12** (M7); **10** (M10)
12	TEP	Efavirenz lamivudine/ abacavir	15 952	495	22	None	K65R, **E138Q,** M184V **M230L**	**28** (M1); **47** (M3); 67 (M12)
13	TEP	Atazanavir/r lamivudine/ abacavir	40	61	13	M41L, A98S, V179I, M184V, T215K	M41L, A98S, V179I, **E138K** M184V, T215K	94 (M4); 119 (M9)
14	TEP	Raltegravir Saquinavir/r	5185	16 657	5	A98G **K103N L100I**	NA	118 (M1); 181 (M4)
15	TEP	Efavirenz TDF/FTC	40	62	12	None	M41I, M184I G190R, **M230I**	248 (M5)
16	TEP	NA	40	50	9	NA	Amplification negative	74 (M6)
17	TEP	Darunavir/r TDF/FTC	50	51	15	NA	None	103 (M9)
18	TEP	Efavirenz TDF/FTC	338	375	22	M41L, V90I, K103N, V108I, V118I, M184V, T215F	M41L, V90I, K103N, V108I, V118I, **E138K,** V179I, M184V, T215F	199 (M1); 232 (M2); 119 (M3); 84 (M4); **29** (M7)
19	TEP	Atazanavir/r TDF/FTC	40	186	9	D67N, K70R, A98AE, K101R, K103N, M184V, K219Q	D67N, K70R, A98G, K103N, V108I, M184V, K219Q	**34** (M1); 175 (M10); 168 (M13)

NP: naive patients; TEP: treatment-experienced patients; TDF: tenofovir disoproxil fumarate; FTC: emtricitabine; NA: not available; cART: combination antiretroviral therapy; RPV: rilpivirine; VL: plasma HIV-1 viral load; VF: virological failure; RT: reverse transcriptase; r: ritonavir. Mutations indicated in bold are specific mutations conferring resistance to rilpivirine; C_{trough} values indicated in bold are suboptimal (< 50 ng/mL);

[a]RPV concentration was collected 12 hours after drug intake.

TABLE 3 RPV trough concentrations

Parameters median (IQR) or *n* (%)	General population (*n* = 343)	Naive population (NP) (*n* = 55)	Treatment-experienced population (TEP) (*n* = 288)
RPV C_{trough}, ng/mL	96 (66–148)	98 (64–141)	96 (66–149)
Inter-subject variability, %	54%	61%	52%
Intra-subject variability, %	27%	29%	26%
Number of RPV C_{trough}	662	112	550
< 50 ng/mL	89 (13.4%)	21 (18.8%)	68 (12.4%)
Number of patients with a RPV C_{trough} < 50 ng/mL	64 (18.7%)	10 (18.2%)	54 (18.8%)
Only one	48 (75%)	4 (40%)	44 (81.5%)
≥ 2	16 (25%)	6 (60%)	10 (18.5%)

C_{trough}: trough plasma concentration; IQR: interquartile range; RPV: rilpivirine.

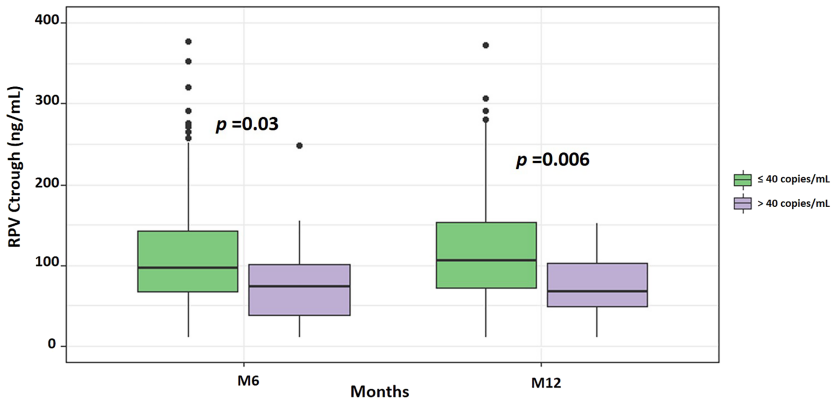

FIGURE 2 Boxplot of rilpivirine trough plasma concentration (RPV C_{trough}) in patients with VL ≤ 40 and > 40 copies/mL at M6 and M12. Comparison of RPV C_{trough} values between patients with VL ≤ 40 (green) and > 40 copies/mL (purple). The boundaries of the box indicate the 25th and 75th percentiles, respectively. The line inside the box represents the median and the whiskers correspond to the 5th and 95th percentiles. Black dots represent outliers

suppressed: 72 ng/mL (39–102; *n* = 18) *vs* 97 ng/mL (68–143; *n* = 265) (*P* = 0.03). Similar results were found at M12: 68 ng/mL (49–103; *n* = 13) *vs* 103 ng/mL (71–153; *n* = 286) (*P* = 0.006) (Figure 2). The proportion of patients with RPV C_{trough} below 50 ng/mL was significantly higher for patients with detectable VL compared with those virologically-suppressed at M6 (28% *vs* 11%; *P* = 0.04), as well as at M12 (31% *vs* 11%; *P* = 0.05). The analysis could not be performed at M24 and M36 due to a too small number of patients with detectable VL (*n* = 8 and *n* = 2, respectively). Figure 3 shows that the optimal RPV C_{trough} cut-off value at M12, which gives the best balance between sensitivity or specificity for efficacy, was 70 ng/mL from ROC curve analysis (AUC: 0.72, sensitivity = 0.75, specificity = 0.62).

4 | DISCUSSION

In the present study, we present the RPV C_{trough} values and antiviral efficacy results of a cohort of both NP and TEP treated with the RPV/FTC/TDF regimen and followed up for 3 years. At 48 weeks (M12), undetectable plasma VL was achieved in more than 80% of patients (85% of TEP and 80% of NP). This result was in agreement with findings in previous cohort studies.[21,22] Moreover, our result was also close to those reported in the ECHO/THRIVE and SPIRIT trials with 84% and 89% of virological success at 48 weeks for NP and TEP, respectively.[3,4,6] Virologic success decreases at week 96 (M24) and week 144 (M36), more significantly in NP, which can be attributed to a higher proportion of patients lost to follow-up and treatment discontinuations in this group. Indeed, in our study, 24% of patients had stopped the treatment, more frequently in NP (30.8% *vs* 22.6%). These results were higher than in other published observational studies reporting discontinuation rates of 13–16% in TEP but with shorter follow-up of 12–16 months.[17,18] In contrast, Sculier et al. reported 25% of discontinuation after a median time of 18.4 months.[22] Finally, our result was lower than the 34% showed by Bernaud et al., where the median time of follow-up was similar (36 months).[26] At M12, the proportion of treatment discontinuation was also higher (8.5%) than reported in clinical trials (3% in ECHO/THRIVE[2,3] and 2.4% in SPIRIT[5]), in line with other cohort studies.[21,22,26] The major reason for treatment discontinuation was toxicity, which was in agreement with previous observational cohort studies. Neuropsychiatric effects were the main reason such as in the study by Gianotti et al.[18] Gastrointestinal disorders are frequently reported as the first cause of treatment interruption. However, in our study, only two cases were observed.

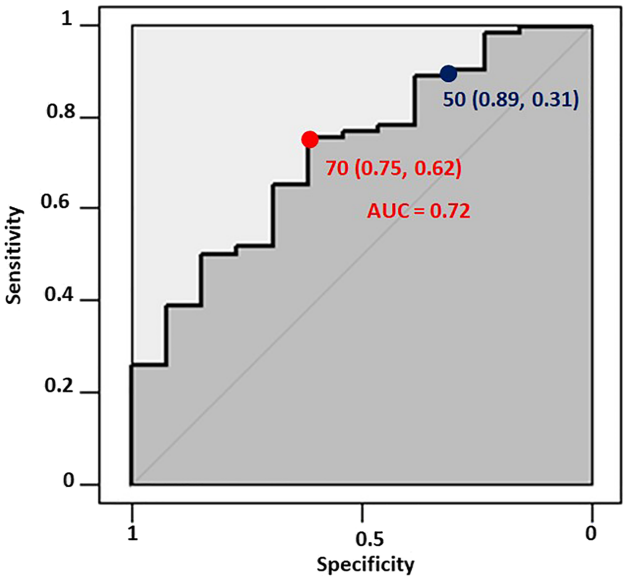

FIGURE 3 ROC curve analysis to identify the optimal RPV C_{trough} cut-off value for achieving virologic response.The true positive rate (sensitivity) is plotted as a function of the true negative rate (specificity) for different RPV C_{trough} cut-off values. The diagonal line represents a sensitivity and specificity of 1. The best balance between sensitivity and specificity was achieved at the cut-off value of 70 ng/mL (sensitivity = 75%; specificity = 62%)

Our results confirm that in clinical practice, side effects lead more frequently to ARV treatment switch.

The rate of VFs observed over 3 years in our study was in accordance with those previously reported in cohort studies ranging from 1.6% to 5.9% at M6 to M12.[20,21,26] Among patients carrying RPV-associated resistance mutations at baseline, only one failed the treatment. This patient should not have received RPV as he presented the K103N + L100I mutations at baseline that confer complete resistance to RPV. Among patients with VF, two had FTC resistance-associated mutations and one harboured mutations associated with an intermediate resistance to TDF, which may have affected treatment efficacy.

The median observed RPV C_{trough} of 96 ng/mL was slightly higher than the value of 74 ng/mL reported at week 48 in the ECHO/THRIVE PK substudy.[27] A higher inter-subject variability was also observed, as expected with real-life data, which may be explained by a strong variability in RPV absorption. The intra-subject variability of 27% was lower than the inter-subject variability reported in the ECHO/THRIVE trials.[28] A suboptimal RPV C_{trough} was found in 19% of patients, which was similar in NP and TEP. This result was lower than the 29% predicted by the Pop-PK model performed in the same context of routine clinical practice.[12] Suboptimal exposure may be the consequence of several factors such as drug–drug interactions (DDI), PK variabilities or adherence difficulties. Thus, in clinical practice, the use of therapeutic drug monitoring (TDM) for ARV is valuable to rapidly detect patients with suboptimal exposure.

A significant relationship between RPV C_{trough} and virological response has been demonstrated at both M6 and M12. Indeed, we highlighted that the median RPV C_{trough} value was significantly lower in patients with detectable VL compared with patients virologically suppressed (−26% at M6 and −34% at M12). Moreover, almost one third of patients with RPV C_{trough} below the 50 ng/mL threshold had detectable VL at M6, which was significantly higher compared with those virologically suppressed. A similar significant trend was observed at M12. These results are consistent with those from the first RPV concentration–response model that we recently published, showing that RPV C_{trough} impacts both the proportion of undetectable patients and the time to obtain virological success.[29]

The optimal RPV C_{trough} cut-off value of 70 ng/mL determined using ROC curve analysis was higher than the currently used 50 ng/mL value, in line with our recently published concentration–response model.[29] Indeed, based on simulations, we had shown that an RPV C_{trough} of 100 ng/mL would be necessary in induction treatment to reach more than 80% of virological success in both NP and non-virologically-suppressed TEP.[29] In the current study, we present analysis of the data from the entire population, including the TEP virologically suppressed at baseline, which could explain that the RPV C_{trough} cut-off found is lower than the previous identified target of 100 ng/mL. From a pharmacological and virological point of view, it is consistent to consider a higher C_{trough} value in induction compared with a maintenance treatment as the intrinsic efficacy required to obtain the undetectability is higher. Therefore, we could consider two thresholds for RPV C_{trough} according to the baseline virologic status of the patients. However, our target for RPV C_{trough} must be interpreted with caution because our study included a small number of patients with detectable VL at baseline.

Overall, 47% of the VF (i.e. eight out of 17 with genotyping data) have developed resistance to RPV. Among these patients, one out of two had at least one suboptimal observed RPV C_{trough} during the monitoring. Reasons for suboptimal RPV plasma exposure observed in half of the VF who developed resistance to RPV may be a poor or variable treatment adherence, food intake failure or unknown DDI. Moreover, two other patients had unexplained high RPV C_{trough} that could also suggest an erratic treatment adherence. Furthermore, the nine other patients who failed without emergence of mutation to RPV always displayed RPV C_{trough} ≥ 50 ng/mL during the follow-up, except one for whom RPV C_{trough} was suboptimal only once during the follow-up. However, if we consider the 70 ng/mL target for RPV C_{trough}, 50% of the patients who failed the regimen, and five out of eight among those who developed resistances to RPV, presented at least one suboptimal RPV C_{trough} throughout their follow-up. These results strengthen the hypothesis of the relationship between suboptimal RPV exposure and drug resistance development.

In conclusion, our study, carried out over a long period of follow-up, highlighted the impact of suboptimal RPV C_{trough} on both virologic response and the emergence of RPV mutations. Moreover, we observed that a significant proportion of patients displayed an RPV C_{trough} below the target cut-off value of 50 ng/mL during the follow-up. Consequently, these results strengthen the use of TDM in real-life contexts to rapidly detect suboptimal exposure and to discuss dose adjustment strategies to reduce the risk of emergence of resistance mutations leading to virologic failure. Moreover, these results, along

with our recently published concentration–response model, suggest that a higher target of RPV C_{trough} might be recommended in clinical practice, particularly in induction strategies.

COMPETING INTERESTS

There are no competing interest to declare.

CONTRIBUTORS

N.N.: PhD student; conceptualization, data acquisition, statistical analysis, interpretation; main writer of the manuscript. M.L.: data acquisition, writing review and editing. N.B.: methodology of the analysis, writing review and editing. F.G.: conceptualization, methodology of analysis, writing review and editing. D.D., C.T.: virologists, data validation, writing review and editing. C.D., S.B., S.M., Y.Y.: recruitment of the patients, writing review and editing. G.P.: pharmacologist, supervision of the analysis, writing review and editing. B.L.: pharmacologist, supervision of the analysis, writing review and editing. C.S.: principal investigator, pharmacologist, conceptualization, methodology, supervision of the analysis, data validation, writing and editing.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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